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**EVALUATION OF THE SQUIRREL MONKEY (SAIMIRI SCIUREUS)  
AS AN EXPERIMENTAL ANIMAL MODEL FOR DYSBARIC  
OSTEONECROSIS**

**James L. Kupper**

**Naval Aerospace Medical Research Laboratory  
Pensacola, Florida**

**7 January 1976**

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER <b>NAMRL- 1221</b>	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) <b>EVALUATION OF THE SQUIRREL MONKEY (<i>Saimiri sciureus</i>) AS AN EXPERIMENTAL ANIMAL MODEL FOR DYSBARIC OSTEONECROSIS</b>		5. TYPE OF REPORT & PERIOD COVERED <b>Final</b>
7. AUTHOR(s) <b>James L. Kupper, Lt Colonel, USAF VC</b>		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS <b>Naval Aerospace Medical Research Laboratory Pensacola, Florida 32512</b>		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS <b>Naval Medical Research and Development Command, National Naval Medical Center Bethesda, Maryland 20034</b>		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS <b>MR000.01.01.7022</b>
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE <b>7 January 1976</b>
		13. NUMBER OF PAGES <b>12</b>
		15. SECURITY CLASS. (of this report) <b>Unclassified</b>
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report)  <b>Approved for public release; distribution unlimited.</b>		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)  <b>Osteonecrosis, Squirrel monkeys, Dysbarism, Decompression</b>		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  <b>In an attempt to develop an animal model for dysbaric osteonecrosis in man, squirrel monkeys were repeatedly exposed to a profile consisting of both hyperbaric and hypobaric pressures. Clinical and subclinical decompression sickness was produced. No clinical, radiologic, or post-mortem evidence of osteonecrosis was discovered during either the 6-month pressure exposure or the 13-month observation period that followed.</b>		

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FOR DYSBARIC OSTEONECROSIS

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Lieutenant Colonel James L. Kupper, USAF, VC



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Naval Medical Research and Development Command  
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NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY  
PENSACOLA, FLORIDA 32512

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## **SUMMARY PAGE\***

### **THE PROBLEM**

To develop an animal model for dysbaric osteonecrosis.

### **FINDINGS**

Squirrel monkeys were repeatedly exposed to a profile consisting of hyperbaric and hypobaric pressures that caused clinical and subclinical decompression sickness in the monkeys. No clinical, radiologic, or post-mortem evidence of osteonecrosis was discovered during either the 6-month pressure exposure period or the 13-month observation period that followed.

### **ACKNOWLEDGMENTS**

The services provided by NAMRL Visual Aids Branch and Veterinary Sciences Division are appreciated. The author is especially grateful to A. L. Armstrong for his diligent efforts in setting up and maintaining the experiment.

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\*The animals used in this study were handled in accordance with the Principles of Laboratory Animal Care established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science, National Research Council.

## INTRODUCTION

Dysbaric osteonecrosis has been described in the literature since late in the last century under a variety of names, i.e., avascular necrosis, aseptic bone necrosis, and caisson bone disease (9). It might be best described histopathologically as aseptic, ischemic osteonecrosis. The disease has been reported in military divers (1,15), low pressure chamber operators (2,7), and aviators (7). The relatively high incidence of specific bone and joint lesions in certain occupations leaves little doubt that it is the result of compression/decompression (10), but the exact pathogenesis is unknown. A high incidence of bone lesions has been reported in individuals with a history of decompression sickness (9), which can be caused by diving or flying alone but particularly by combinations of both (4,6).

Attempts to produce the syndrome in experimental animals have had limited success. Dogs (11) and guinea pigs (5) are apparently refractory. Lesions have been reported in swine (14), but, this species is large, difficult to handle, expensive to maintain, and phylogenetically distant from man. The squirrel monkey is especially suitable for biomedical research because of its small size, tractability, easy maintenance, and ready availability (8). (The latter consideration is uncertain at this time, however, due to export restrictions by the countries of origin.)

The purpose of this study was to provide a suitable animal model for dysbaric osteonecrosis by subjecting squirrel monkeys, Saimiri sciureus (2), to a combination of hyperbaric and hypobaric pressures and detecting radiographically the bone changes associated with the disease.

## PROCEDURE

### SUBJECTS AND APPARATUS

Thirty male Columbian squirrel monkeys (Saimiri sciureus) were used in the experiment.

Small Plexiglas cylinders, each divided into two compartments by a solid partition, served as primary enclosures for two animals. These cylinders were fitted with breathing-gas inlet pipes on the side and several small gas-escape ports in the ends. In this way, relatively high flow rates could be maintained around the animals and rebreathing of exhaled gas minimized.

A 180-liter hyperbaric chamber that would accommodate five Plexiglas cylinders was used for overcompression (Figure 1). Compressed air was supplied from a high-pressure cylinder bank. Air entering the chamber was directed by a flexible manifold through the Plexiglas cylinders containing the animals. A flow rate of 15 liters/minute was maintained throughout the hyperbaric exposure.



Figure 1  
Plexiglas Primary Enclosures in Small Hyperbaric Chamber

A large man-rated chamber was used for the hypobaric profile. The small Plexiglas cylinders were placed on a rack (Figure 2) with 100% oxygen provided instead of compressed air.

Radiographs were taken with a 300 mA general Electric\* machine and using 25 x 30 cm Spectroline+ cassettes with intensifying screens. The animals were restrained in dorsal recumbency on the cassettes by a combination of straps and wooden blocks.

A 10 mHz Doppler ultrasonic flowmeter and transcutaneous probe were used to detect intravascular gaseous embolism in major thoracic or abdominal vessels. The Doppler ultrasonic flowmeter measures blood velocity from an extra-vascular position by detecting frequency shifts incurred by ultrasound reflected by passing blood cells. Gas bubbles in blood have been noted to be even more effective reflectors of ultrasound, and thus are readily detected and distinguishable from normal blood flow (13).

## METHOD

Five additional squirrel monkeys were used in a pilot study to develop a pressure profile that would produce intravascular bubbles in the absence of clinical signs. Intravascular gas embolism is a feature of decompression sickness and is believed to be responsible for many of the symptoms (12). Various hyperbaric and hypobaric pressures and durations, as well as the interval between each, were tested. A combination that consistently produced bubbles in more than 50% of the five monkeys consisted of pressurization to 2.4 kg/cm<sup>2</sup> (80 feet sea water equivalent) for 3 hours followed immediately by 1 hour at 0.58 kg/cm<sup>2</sup> (15,000 feet altitude equivalent). This profile was used on the experimental groups.

The thirty conditioned and stabilized experimental squirrel monkeys were divided into three groups of 10 each. Group A was designated as control and was subjected to all phases of the experimental protocol except for the pressure changes. Groups B and C were exposed once a week to the pressure profiles for 26 weeks. All groups were retained and monitored clinically and radiographically for osteonecrotic anomalies. Radiographs were taken at approximately 3-month intervals.

Two alterations in the protocol occurred. Hematologic determinations had originally been proposed, but experience with other squirrel monkeys had shown collection of blood samples to be a stressful procedure in this species. In addition, the only consistently accessible peripheral vessel is the femoral vein. Venipuncture attempts in this area occasionally result in invasion of the femoral artery. While this usually causes only minimal and reversible damage to the artery, hematomas and partial

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\* General Electric Company, Milwaukee, Wisconsin  
+ Spectronics Corporation, Westbury, N. Y.



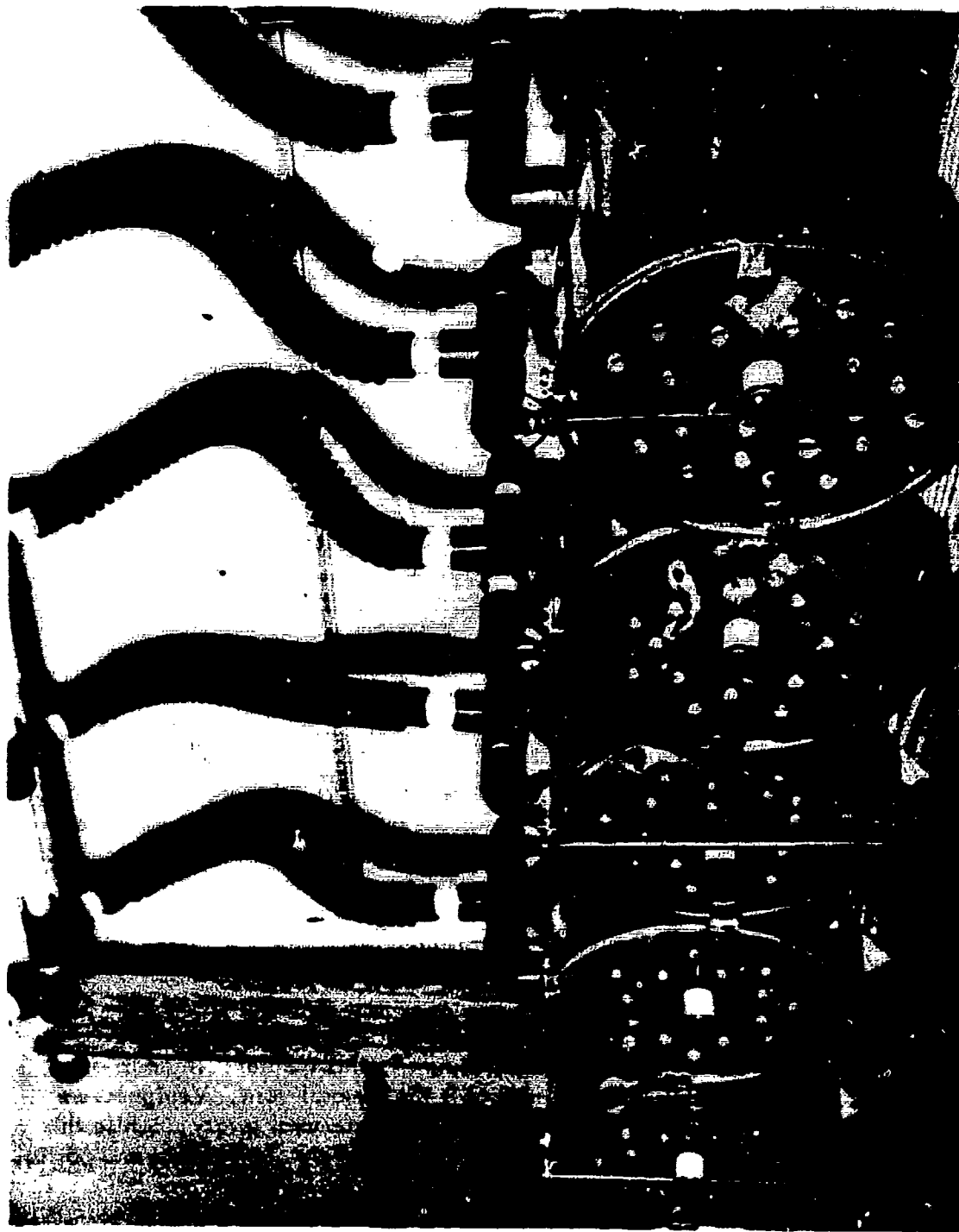


Figure 2  
Rack of Cylinders in Low Pressure Chamber, with Flexible Oxygen Hoses to Each Monkey

occlusions can result. The present investigation was concerned with a bone lesion in which impaired blood supply has been incriminated. The stress of the sampling procedure and risk of vascular impairment to the pelvic limb led to a decision to omit hematologic determinations. A second change in the protocol involved not sacrificing one group of the animals at the end of the dysbaric exposures for histopathologic study. In the complete absence of radiologic evidence of bone changes, a decision was made to retain all groups for a longer period to allow lesions to develop.

## RESULTS AND DISCUSSION

No clinical, radiologic, or post-mortem evidence of dysbaric osteonecrosis was discovered during either the 6-month pressure exposure or the 13-month observation period that followed.

Five of the 30 animals have died since the beginning of the experiment. One monkey from Group C collapsed an hour after his 24th exposure to the pressure profile. Post-mortem findings were consistent with decompression sickness; i.e., subcutaneous emphysema and gaseous emboli in pulmonary and cerebral vessels. The second monkey belonged to control Group A. During the last week of pressure exposures it developed tremors and dyspnea while in the Plexiglas cylinder in the hypobaric chamber at sea-level pressure. The rectal temperature reached 41.2° C during resuscitative efforts and then dropped quickly to subnormal, at which time terminal convulsions occurred. Absence of significant gross or microscopic lesions tends to support a diagnosis of thermoregulatory malfunction. A monkey from Group B was found dead in its cage one month after the pressure exposures had been completed. Post-mortem examination was unremarkable. The fourth animal (Group C) was discovered moribund on the bottom of its cage and expired while being examined. Post-mortem examination revealed a perforated pyloric ulcer with serous fluid and gastric contents in the abdominal cavity. Another animal from Group B died during a surgical procedure to remove an enlargement from the right arm. Necropsy findings include subcutaneous nocardial granulomata and pulmonary fibrosis. The exact relationship between the pulmonary fibrosis and death under anesthesia is difficult to establish.

It must be concluded that the squirrel monkey is not highly susceptible to dysbaric osteonecrosis. Whether the species is refractory to the disease cannot be established from the present study. Funding has not been secured to retain these animals and monitor them for an extended period; however, efforts will be made to assign the exposed animals to other than terminal experiments and maintain at least an annual radiographic surveillance program.

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